Syntheses, π-stacking interactions and base-pairings of uracil pyridinium salts and uracilyl betaines with nucleobases†

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Reaction of 6-chlorouracil with 4-(dimethylamino)pyridine, 4-methylpyridine, and pyridin-4-yl-morpholine yielded pyridinium-substituted uracils as chlorides which were converted into pyridinium uracilates by deprotonation. These heterocyclic mesomeric betaines are cross-conjugated and thus possess separate cationic (pyridinium) and anionic (uracilate) moieties. Calculations and X-ray single crystal analyses were performed in order to characterize these systems and to compare the salts with the betaines. ^{1}H NMR experiments in D_2O proved π-interactions between the uracilyl betaines and adenine, adenosine, as well as adeninium. No π-stacking interactions were detected between the betaines and guanosine. The acidic N8-H group of the uracil pyridinium salts caused acid–base reactions which were observed in parallel to π-stacking interactions. Self-complementarity of the modified uracils was detected by ^{1}H NMR experiments in DMSO-d_6 and electrospray ionisation mass spectrometry (ESIMS). *Ab initio* calculations predicted base-pairings of the modified uracils with adeninium, cytosine, and guanine. Several geometries of hydrogen-bonded associates were calculated. Hoogsteen pairings between the uracil-4-(dimethylamino)pyridinium salt and adeninium, as well as associates between the corresponding betaine plus cytosine, and the betaine plus guanine were calculated, and the most stable conformations were determined. In the ESI mass spectra, prominent peaks of associates between the modified uracils and adeninium, cytosine, cytidine, guanosine and d(CpGp) were detected.

Introduction

Nature produces a surprisingly large variety of conjugated molecules which can exclusively be represented by dipolar canonical formulae. These molecules, the so-called mesomeric betaines (MB), delocalize an even number of positive and negative charges within a common π-electron system. Numerous mesomeric betaines were identified as alkaloids, and it was recognized that a smaller number of these systems serve as modified nucleobases.[1] The degree of charge-separation in mesomeric betaines is modulated by the type of conjugation, and, as a consequence, four distinct types of this class of compounds were defined: (i) conjugated (CMB), (ii) cross-conjugated (CCMB), (iii) pseudo-cross-conjugated heterocyclic mesomeric betaines (PCCMB) in addition to (iv) ylides such as N-oxides and N-ylides as an additional subclass of CMBs.[2] The type of conjugation significantly influences the biological, chemical, and physical properties of these molecules.[3] Examples of naturally occurring mesomeric betaines are the alkaloids Fumonisin (CMB),[4] Pyridinoline (CMB),[5] Neooxygambirtannine (CMB),[6] Trigonellin (CCMB),[7]

Pyridinebetaine A and B (CCMB),[8] Nigellicine (PCCMB)[9] and Homarine (PCCMB).[10] Among the exceptional number of post-transcriptionally modified nucleosides, 7-methylguanosine (m[7]G) **1**, 2,7-dimethylguanosine (m[2,7]G) **2**, and 2,2,7-trimethylguanosine (m[2,2,7]G) **3** are members of the class of conjugated mesomeric betaines (Scheme 1). They were isolated from distinct types of RNA (ribosomal RNA,[11] archaeal, bacterial , and eukaryotic transfer-RNA,[12] sn,[13] viral[14] and messenger RNA[15]). These compounds undergo non-standard base-pairings such as m[7]G=G≡C,[16] and unusual π-stacking interactions such as the intercalation of adenine into m[7]G and G to stabilize the tertiary structures of RNA.[17] 7-Methylguanosine, **1**, which was also isolated as an alkaloid from the marine sponge *Geodia gigas*,[18] forms furthermore the 5′-capping structure of eukaryotic messenger RNA and is joined to the RNA through an unique triphosphate bridge Gp(5′–5)ppN. The m[7]G(5′)ppp(5′)N mRNA cap is recognized in the splicing of the first intron in nascent transcripts, transport of mRNA through the nuclear envelope,[19] and translation of the message by ribosomes.[20] Thus, formation of a mesomeric betaine from guanosine must present a ligand that is distinct from the large pool of unmethylated guanine nucleotides in cells.[21]

The atomic structures of two specific m[7]G-protein complexes have been determined. In one complex the mesomeric betaine is stacked between two tryptophan residues and a glutamate side chain that forms a hydrogen-bond to the purine ring.[22] Studies on model compounds suggest a complex between the positively charged π-ring of m[7]G and the electron rich indole moiety of trytophan.[23] In the other complex, m[7]G performs π-stacking interactions to tyrosine and phenylalanine.[24] Results of studies

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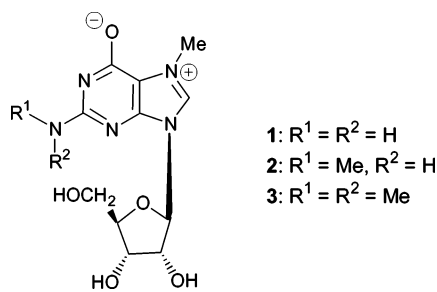
Scheme 1

Scheme 2

on structural requirements for the specific recognition of m⁷GDP suggest that a complicated pattern of both orientation and identity of stacking residues are necessary for the selective binding.[21]

As part of an ongoing project we are interested in modified nucleobases that are members of the class of heterocyclic mesomeric betaines.[25–28] We present here the syntheses of uracilium salts and their corresponding heterocyclic mesomeric betaines, pyridinium uracilates. We performed calculations to characterize the charge-separated ground state of these substances and studied intermolecular interactions. In our compounds, the uracil represents the anionic partial structure of a mesomeric betaine which is stabilized by a pyridinium substituent in cross-conjugation. We report our results of NMR measurements, electrospray ionization mass spectrometry (ESIMS), X-ray single crystal analyses, and calculations on base-pairing properties of the modified uracils to adenine, guanine, and cytosine, as well as to the DNA model compound d(CpGp).

Results and discussion

Syntheses and classifications

The syntheses of the cross-conjugated mesomeric betaines **7**, **9**, and **11** are depicted in Scheme 2. The synthesis started from trichloropyrimidine **4** which was converted into 6-chlorouracil **5** according to known procedures.[29] Substitution of the chloro substituent by 4-(dimethylamino)pyridine, 4-methylpyridine, and 4-pyridin-4-yl-morpholine yielded the water-soluble uracilium salts **6**, **8**, and **10**, respectively.

Deprotonation with Amberlite IRA-400 in its hydroxy form gave the mesomeric betaines **7**, **9**, and **11**. Whereas **7** and **11** were formed in almost quantitative yields, the betaine **9** was obtained in only 26% yield. This presumably is due to deprotonation of the acidic methyl group and side-reactions on the anion exchange resin.

On betaine formation, nearly all resonance frequencies shift considerably upfield. For example, the singlet of 12-H of **8** shifts from 6.25 to 5.71 ppm on formation of **9**, so that this resonance frequency can serve as a reliable indicator to observe the acid–base properties of the modified uracils. Furthermore, the resonance frequency of 12-H proved to be a very reliable tool for the detection of π-stacking interactions and base-pairing properties, as it forms a sharp singlet which is not overlapped by the signals of added nucleobases. As presented in Fig. 1, the Watson–Crick binding sites are involved in the delocalization of the negative charge in the pyrimidine rings. Deprotonation of the salts **6**, **8**, and **10** converts the acceptor–donor motifs (AD) C2=O/N1–H into
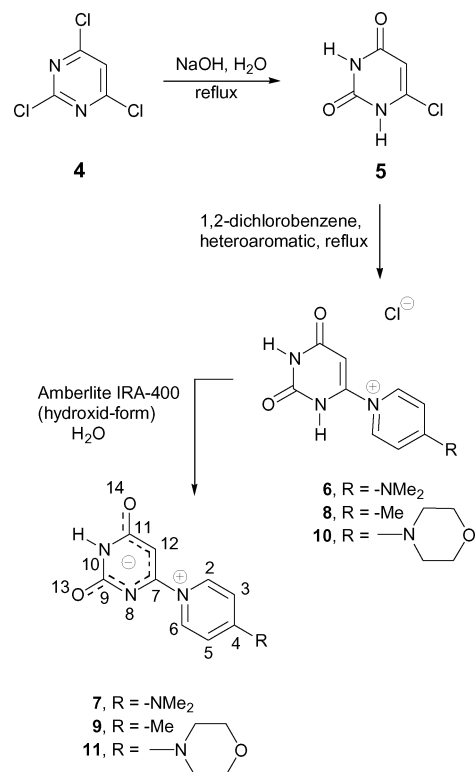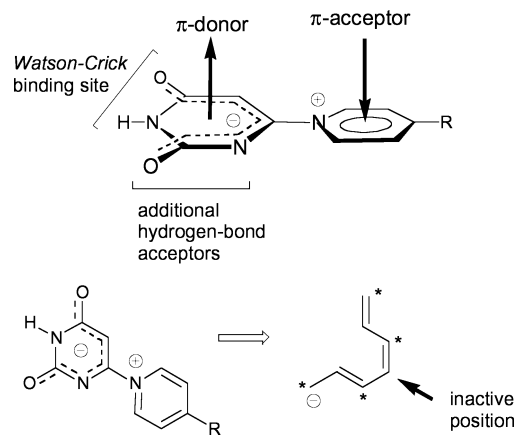

Fig. 1 Classification of the betaines.

acceptor–acceptor motifs (AA) in **7**, **9**, and **11**. Furthermore, in view of the characteristics of heterocyclic mesomeric betaines, π-donor (pyrimidine) and π-acceptor (heteroarenium) moieties of the pyridinium uracilates can be expected. A precondition for this is cross-conjugation between the positive and the negative partial structures. Thus, the positive fragment is joined to the anionic partial structure (the uracil) through an unstarred atom, *i.e.* a nodal position of the isoconjugated equivalent, the 1,3,5-heptatrienyl anion. This position serves as an isolator between the charges, which are therefore strictly delocalized in the separated parts of the molecule. These features are characteristic for cross-conjugated heterocyclic mesomeric betaines.[1–3]

Profound differences between the salts and the betaines became obvious by calculations of two model compounds, the 4-(dimethylamino)pyridine-substituted uracils **6** and **7**. Calculations

on the uracil pyridinium salt **6** in an aqueous environment led to a twisted molecule with a torsion angle of 53.55° between the pyrimidine and the pyridinium ring. Natural bond orders (NBO) indicate that the pyridinium ring adopts a quinoid structure. The NBO values for C2–C3, C5–C6 and C4–N are 1.87, 1.86 and 1.96, respectively. The calculated bond lengths reinforce this fact. Thus, the optimized values for the same bonds are, respectively, 136.2, 136.2 and 133.6 pm, while those of the C3–C4 and C4–C5 are both 142.8 pm. The highest occupied molecular orbital (HOMO: −0.25496 eV) as well as the lowest unoccupied molecular orbital (LUMO: −0.08484 eV) are located in the pyrimidine as well as in the pyridinium ring (Fig. 2). TD calculations on the B3PW91/6-31G**/PCM optimized structure predicted the lowest energy transitions at 294.4 nm (4.212 eV) and 279.2 nm (4.441 eV), with oscillator strengths of 0.439 and 0.025, respectively. The lowest transition energy is usually compared with the HOMO–LUMO energy gap since this electronic transition is described as the promotion of a single electron from HOMO to LUMO.[30] However, this comparison clearly fails for this molecule, as the computed energy for the first electronic transition is significantly higher than the calculated HOMO–LUMO gap. This fact is attributed to the reduced interelectronic interaction between the single one-electron excitation.[31] It also indicates that this transition has to be described as a linear combination of single one-electron promotions between a set of frontier orbitals. For this molecule, the TD/PCM calculation assigned the transition at 4.441 eV to the HOMO to LUMO (62%) and the HOMO-1 to LUMO (21%) excitations, together with other minor contributions.
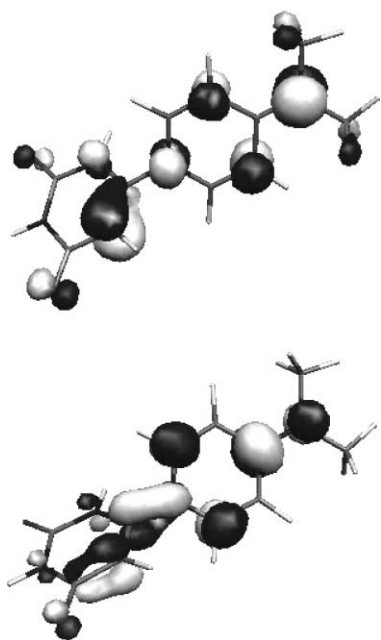

Fig. 2 HOMO (above) and LUMO (below) of **6**.

By contrast, the torsion angle of the betaine **7** was calculated to be 31.16°. The HOMO is essentially located in the pyrimidine ring (−0.21224 eV) and the LUMO is essentially located in the pyridinium ring (−0.06889 eV) (Fig. 3). As predicted by the concept of cross-conjugation in heterocyclic mesomeric betaines, C7 is a nodal position of the HOMO and thus serves as an isolator between the charges (cf. Fig. 1). This is reflected in the calculated
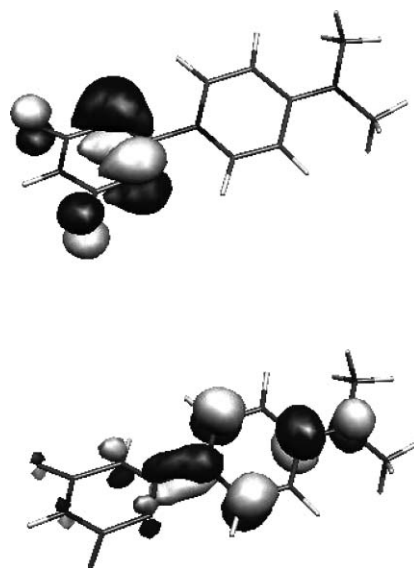

Fig. 3 HOMO (above) and LUMO (below) of betaine **7**.

Table 1 Atomic charges with hydrogens summed into heavy atoms of cation **6** and betaine **7**. For numbering, cf. Scheme 2

Atom	Charge in **6** (a.u.)	Charge in **7** (a.u.)
N1	−0.554545	−0.547306
C2	0.389803	0.369394
C3	0.000754	−0.021063
C4	0.432212	0.426923
C5	−0.004468	−0.023730
C6	0.388206	0.377863
C7	0.541854	0.487072
N8	−0.274277	−0.661682
C9	0.808940	0.690852
N10	−0.279934	−0.301851
C11	0.654995	0.607203
C12	−0.027774	−0.132859
O13	−0.557887	−0.639064
O14	−0.573508	−0.646389
N15	−0.513083	−0.518514
C16	0.279894	0.266522
C17	0.279881	0.266631

atomic charges, which indicate the delocalized negative charge in the pyrimidine ring (Table 1). The lowest energy transitions were rather different to those calculated for the cation, namely at 371.8 nm (3.344 eV) and 309.8 nm (4.003 eV), with oscillator strengths of 0.061 and 0.004, respectively. As a consequence, the difference between the lowest transition energy and the HOMO–LUMO energy gap results 3.2004 eV, which is quite lower than for the uracil salt **6**, 4.0416 eV. Further B3PW91/6-31G** gas phase calculations, performed for both the uracil salt **6** and the betaine **7**, indicated that the solvent effect is also significantly stronger in the betaine than in the salt. Thus, the predicted energies of the first electronic transitions were 3.6569 and 2.0794 eV, respectively, which involves an energy decrease of 1.2643 eV for betaine **7** with respect to the TD/PCM result for the same molecule, while this deviation was only 0.5548 eV for the uracil salt **6**. NBO values are nevertheless similar to those obtained for the uracil salt **6**, which is also supported by the optimized bond lengths.

Electrostatic surface potentials calculated for the uracil pyridinium salt **6** and the betaine **7** show significant polarization of

the π-systems due to electron-donating uracil moieties and the electron-withdrawing pyridinium cations (Fig. 4). This polarization is more significant in the betaine **7** where the cross-conjugation tends to localize the charge into separate parts. On the other hand, the protonation in the salt **6** provokes a distorted conformation between the uracil and the pyridinium rings. As a consequence, the C2=O/N1–H group acquires a stronger donor character as in the betaine molecule **7**.
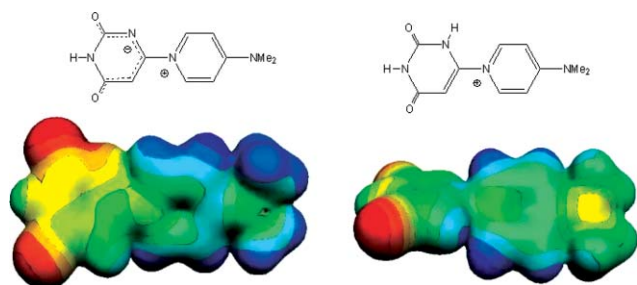

Fig. 4 Electrostatic surface potentials calculated for the 4-(dimethylamino)pyridine substituted uracils **6** (right) and **7** (left) using *ab initio* calculations in an aqueous environment. The shortage of electron density is shown in blue and the relatively high electron density is shown in red. These calculations and the color scaling used are meant for qualitative comparisons only.

The structure of uracilium salt **10** was elucidated by a single crystal X-ray analysis. Suitable single crystals were obtained by slow evaporation of **10** in water. The molecular drawings and the crystallographic numbering of the molecule is shown in Fig. 5. In the single crystal, the pyridinium ring is twisted out of the plane of the uracil moiety; the corresponding dihedral angle C3–C4–N7–C8 is −48.6(2)°. Characteristic bond lengths are presented in Table 2. Several hydrogen bonds were determined in the elemental cell. Thus, O2 (crystallographic numbering) forms a hydrogen bond to the water of crystallization; the other hydrogen atom of this water molecule forms a hydrogen bond to the chloride anion. N5–H also forms a hydrogen bond to a chloride. Two molecules of the uracilyl salt are connected by a hydrogen bond between N1–H of one uracil to the oxygen atom O16 of the morpholine moiety of another molecule. Additional molecular drawings are presented in the ESI.†
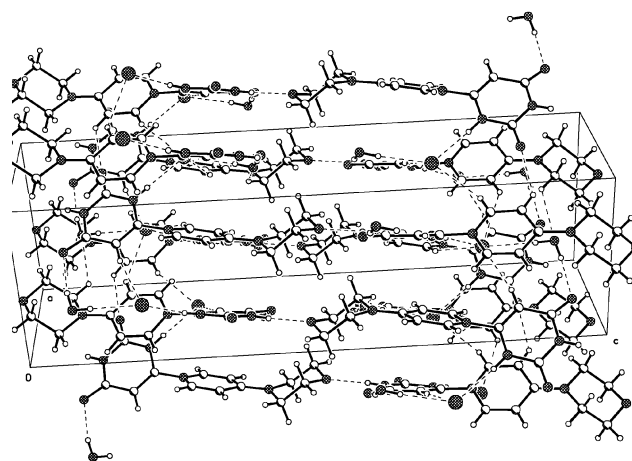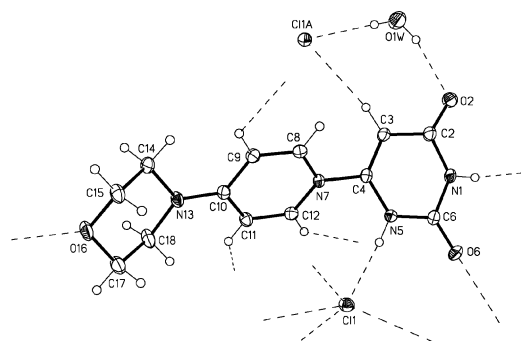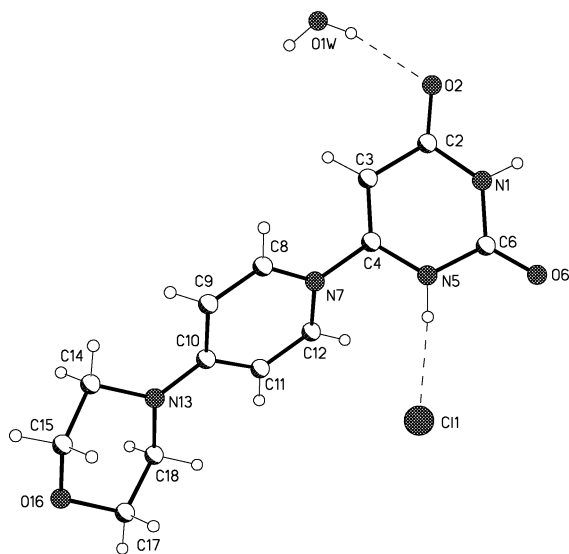

Fig. 5 Molecular drawings of **10**.

π-Stacking interactions

The formation of π-stacks in water is known to be an isodesmic process.[32] Within the π-complexes, the distance between the individual molecules is approximately 340 pm, and amino, imino, and carbonyl groups are often located above aromatic systems of the π-stacking partners.[33] Frontier orbital interactions,[34] electrostatic interactions, solvation and inductive effects such as CT-[23] and EDA-effects, as well as a combination of hydrophobic, electrostatic, and van der Waals interactions[21,35] are regarded as contributors to the binding energy of stacked nucleobases and related compounds. It was discussed that the LUMO energy of m[7]G is significantly lowered due to methylation of N7, so that an electron donation from electron-rich π-ring systems is facilitated.[36] In addition, Coulombic interactions were discussed in this context,[37] calculations were carried out,[38] and model substances, *e.g.*, for the molecular recognition of adenine derivatives in water were developed.[39]

As outlined in Fig. 1, the modified uracils encouraged us to examine π-stacking interactions and hydrogen bonding capabilities. First, we performed concentration dependent ^{1}H NMR experiments in D_2O. Whereas the resonance frequencies of the salt **6** in D_2O remained virtually unchanged on dilution of a concentrated solution, the signals of the picoline derivative **8** and of the morpholinopyridine derivative **10** displayed upfield shifts under analogous conditions[40,41] which proved vertical interactions.

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Table 2 Calculated values and selected results of the single crystal X-ray analysis of **10** (crystallographic numbering)

| Bond lengths (calculated/found)/pm		Bond angles (calculated/found) (deg)		Dihedral angles (calculated/found) (deg)	
N1–C2	140/1.385(2)	N1–C2–C3	114/115.0(1)	N1–C2–C3–C4	−1.7/3.3(2)
C2–C3	145/1.445(2)	N1–C2–O2	121/119.5(1)	C6–N1–C2–O2	1.2/175.5(1)
C3–C4	135/1.339(2)	C2–C3–C4	119/117.9(1)	C2–C3–C4–N7	0.1/−177.6(1)
C4–N5	137/1.359(2)	C3–C4–N5	123/123.9(1)	C3–C4–N7–C8	52.5/−48.6(2)
N5–C6	139/1.375(2)	C3–C4–N7	122/122.1(1)	N1–C6–N5–C4	0.8/4.0(2)
C6–N1	138/1.376(2)	C4–N5–C6	123/121.6(1)	N7–C8–C9–C10	−0.3/0.3(2)
C2–O2	123/1.232(2)	N5–C6–O6	122/122.8(1)	C9–C10–N13–C18	−8.3/−174.6(1)
C6–O6	122/1.219(2)	C4–N7–C8	120/120.4(1)	C10–N13–C14–C15	27.2/−132.0(1)
C4–N7	142/1.434(2)	N7–C8–C9	121/121.4(1)		
N7–C8	137/1.366(2)	C9–C10–N13	122/122.0(1)		
C10–N13	134/1.344(2)	C10–N13–C14	122/123.6(1)		

Upfield shifts were also observed on dilution of concentrated D_2O solutions of the betaines **7** and **11**. As already mentioned, it proved to be advantageous to observe the resonance frequency of 12-H, as it forms a sharp, not H/D-exchangeable singlet which is not overlapped with other NMR signals. As an example, dilution of a 25.71 mmol L^{-1} solution of **11** in D_2O to 2.57 mmol L^{-1} caused an upfield shift of the resonance frequency of 12-H from 5.589 to 5.723 ppm. The solubility of the picoline derivative **9**, however, proved to be insufficient for measurements in D_2O, as the maximum concentration is only 6.86 mmol L^{-1}.

With these informations in hand, we examined chemical shift changes of the modified uracils at given concentrations on addition of equimolar amounts of the nucleobases adenine (ade), adenosine (ado) and guanosine (guo). Unfortunately, guanine is essentially insoluble in water at pH 7, so that we were prevented from examinations of this nucleobase. Literature-known chemical shift differences of the thymine–adenine π-stack are 3 to 216 ppb to the upper field of 6-H of the pyrimidine nucleobase,[42] and 0.10 ppb to the lower field of the resonance frequencies of the purine system. In accordance to this, the signal of 12-H of the uracilyl betaine **7** shifted significantly to the upper field on mixing an aqueous solution with adenine (ade). The chemical shift differences were −140 ppb in comparison to the concentrated solution in D_2O (Table 3, entry IV) and −250 ppb in comparison to the molecule in a highly diluted solution. By dilution to less than 1 mM solutions, the chemical shifts of "monomeric" species can be measured.[43] Thus, the differences were more than twice as large as observed for the natural nucleobase uracil, the resonance frequencies of which shift 60 ppb to the upper field on addition of equimolar amounts of adenine. In parallel, the signals of adenine shifted by

the factor 4 to lower field. As displayed in Table 3, similar results were obtained on studying 1 : 1 mixtures of **7** with adenosine (ado) (Table 3, entry V), although the effect is much smaller. Guanosine (guo), however, caused only very small shift differences so that no π-stacks were proved under these conditions (Table 3, entry VI).

The spectroscopic properties of the salt **6** in the presence of adenine, adenosine, guanine and guanosine are also influenced by the acidic N8–H proton. Thus, equilibria between protonated and deprotonated species according to Scheme 3 were formed, so that in general π-stacking interactions between all species depicted in Scheme 4 had to be taken into consideration. Due to a considerable lower LUMO energy,[44] adeninium (adeH+) is known to form more stable π-complexes with π-donor molecules than adenine itself.

Scheme 3
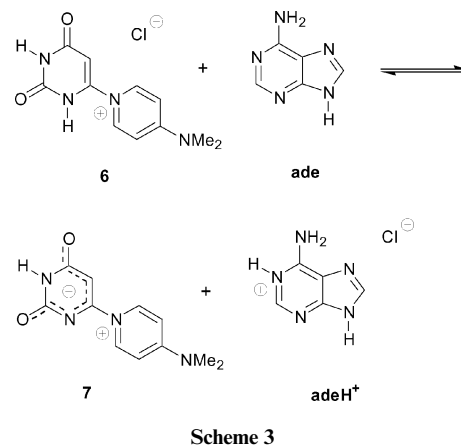
Table 3 Chemical shift changes $\Delta\delta$ to the lower (+) and upper field (−) in relation to the pure compounds in the same concentration on addition of equimolar amounts of adenine (ade), adenosine (ado), and guanosine (guo). Values are give in ppb (parts per billion: 1000 ppb = 1 ppm. Concentration: 10 mM L^{-1} in D_2O, temperature: 25 °C, $\delta(D_2O)$ = 2.500 ppm

			$\Delta\delta$ of the uracils			$\Delta\delta$ of the purines	
	Entry	Base	12-H	2/6-H	3/5-H	2-H	8-H
6	I	ade	−190	120	−110	170	190
	II	ado	−110	80	−65	160	130
	III	guo	−20	3	−20	—	10
7	IV	ade	−140	−50	−140	40	45
	V	ado	−90	−25	−65	50	40
	VI	guo	−25	−10	−20	—	−90
6 → 7	VII	blind	−135	125	−62	—	—
ade → adeH+	VIII	probes	—	—	—	340	310

ade ← 6

adeH⁺← 6

ade ← 7

adeH⁺← 7
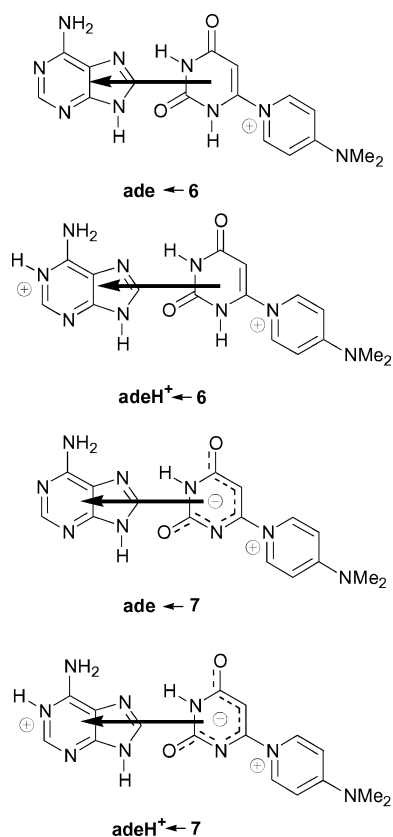
Scheme 4

Before we started the NMR examinations, we prepared adeninium chloride (adeH⁺Cl⁻) and adeninediium dichloride (ade(H⁺)₂2Cl⁻) by addition of equimolar amounts of hydrochloric acid to aqueous solutions of adenine. The structure of the latter mentioned dicationic species was unambiguously elucidated by an X-ray analysis; all the moieties are lying on mirror planes. The molecular drawing is shown in Fig. 6. The structural data which we obtained are identical to those already reported in the literature.[45] Suitable single crystals were obtained by slow evaporation of adenine in 5% aqueous HCl. By resolving the single crystals, the chemical shifts of adeninediium and adeninium were assigned and
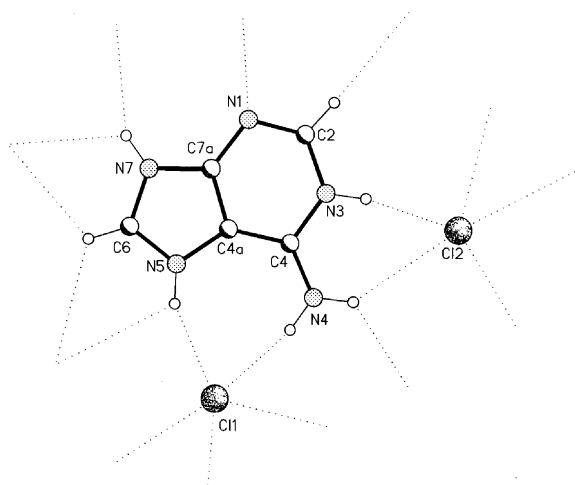

Fig. 6

relevant chemical shift differences could be determined (Table 3, entry VIII).

On mixing adenine (ade) with the salt **6**, 12-H and 3/5-H of **6** were characteristically shielded due to betaine formation, whereas the resonance frequencies of adenine shifted to lower field due to partial protonation (Table 3, entry I). This acid–base reaction was furthermore confirmed by UV measurements: The π–π*-transition of adenine shifted characteristically from $\lambda_{max} = 260.40$ to 268.60 nm due to protonation. However, the chemical shift differences of **6** were much larger than expected for conversion to betaine **7** (Table 3, entry VII). Correspondingly, the shift differences of adeninium to adenine were much smaller than expected (Table 3, entry VIII). These results strongly support the idea of an interplay between two effects (i) acid–base reaction and (ii) π-stacking interactions. The spectroscopic results thus clearly hint at measurable concentrations of π-stacks between **7** and adeninium adeH⁺ in the reaction mixture.

Additional ¹H NMR measurements in D₂O moreover clearly indicated the existence of π-interactions between the salt **6** and adeninium (adeH⁺) in spite of the equal charges of the stacking partners. It is apparent that the chemical shift changes of the pyridinium protons were significantly larger than those of 12-H. Thus, 2/6-H and 3/5-H of **6** shift by −163 and −49 ppb to the upper field, respectively, on addition of equimolar amounts of adeH⁺, whereas the resonance frequency of 12-H shifts by only −20 ppb. This observation hints at influences of energy as well as the geometry of the HOMO of **6**. The aforementioned calculations indeed indicated that the HOMO of **6** possesses a large coefficient at the pyridinium atom. ¹H NMR spectroscopy as well as UV spectroscopy clearly showed that no neutralization of the cation **6** was observable with adenosine which is less basic than adenine (Table 3, entry II) [pK_a(ade) = 4.20; pK_a(ado) = 3.50]. On addition of guanosine guo, no chemical shift changes were observable (Table 3, entry III) [pK_a(guo) = 9.42].

Similar results were obtained on examination of the uracilium salts **8** and **10**. On mixing D₂O solutions of **8** and **10** with equimolar solutions of adenine, considerable upfield shifts of 12-H were observable.[46,47] Adeninium adeH⁺ plus **10** also resulted in an upfield shift, thus proving π-stacking interactions between these species in D₂O.[48]

Hydrogen bonding

The first hint at unusual base-pairing properties of the modified uracils is their self-complementarity. An X-ray single crystal analysis showed that the Watson–Crick binding site is not involved in the self-association of **7** to **7 = 7**, but C2=O and N3–H, forming a centrosymmetric dimer (Scheme 5).[26]

7=7
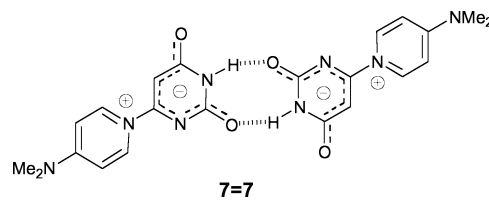
Scheme 5

Self-complementarity in solution was proved by ¹H NMR experiments in anhydrous DMSO-d₆ at different concentrations.

On dilution of concentrated solutions of the cations and betaines **6**, **7**, **8**, **9**, and **10** all resonance frequencies shift upfield in accordance with horizontal interactions in this solvent. As example, the curve of the picoline derivative **8** is presented in Fig. 7. The solubility of betaine **11**, however, proved to be insufficient for satisfying measurements. All compounds are insufficiently soluble in less polar solvents.
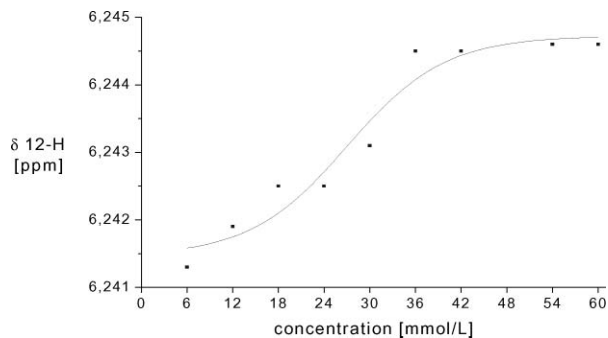

Fig. 7

In the electrospray ionisation mass spectra (ESIMS), the dimers form prominent peaks at 0 V fragmentor voltage. Mixtures of all compounds give rise to combinations of homo- and hetero-intermolecular base pairings. Thus, in the ESI mass spectrum of a 1 : 1 mixture of **6** and **10** peaks of the individual molecules at m/z 233.1 [**6**]$^+$ and 275.1 [**10**]$^+$, as well as homo-intermolecular pairs of [**6** + **7**]$^+$ at m/z 465.1 and [**10** + **11**]$^+$ at m/z 549.2 and hetero-intermolecular adducts of [**6** + **11**]$^+$ or [**7** + **10**]$^+$ at m/z 507.1 (Scheme 6, Table 4) are detectable.
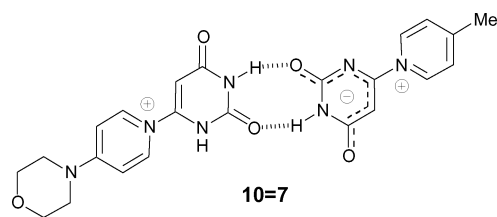
Scheme 6

We next turned our attention to the base-pairing properties of the modified uracils. NMR titrations clearly show, that no hydrogen bonds between uracilylbetaine **7** and adenine, adenosine, cytosine, guanine, and guanosine can be observed in DMSO-d_6 at room temperature. One reason could be the very limited solubility of **7** which does not exceed 4 mM per L solvent. This behaviour is analogous to the natural pyrimidine nucleobases under analogous reaction conditions.[42]

Even the extremely mild electrospray ionization (ESI) technique, which proved to be highly valuable for the detection of oligonucleotides, proteins, enzyme–substrate and enzyme–product complexes,[49] was not able to detect base pairs between the uracil derivatives **8** and **10**, respectively, and adenine.

However, the measurements performed starting from equimolar solutions of **6** or **7** and adenine in acetonitrile–water (9 : 1) at 0 V fragmentor voltage displayed peaks of the monomeric species of **7** at m/z 233.1 (100%) and of **7** = **7** at m/z 465 (20%)—in accordance with the aforementioned self-complementarity of

Table 4 Results of ESIMS measurements. Spectra were taken from solutions of the salts **6**, **8**, and **10** in water–acetonitrile (9 : 1). The samples were sprayed (35 psig, solvent flow 0.6 mL min^{-1}) from water–acetonitrile (9 : 1) at 0 V fragmentor voltage, 4000 V capillary voltage, and 300 °C drying gas temperature (N$_2$). ade = adenine; cyto = cytosine; cyti = cytidine; guo = guanosine

|	Pure samples	Ade	Cyto	Cyti	Guo	d(CpGp)
6	233.1 [**6**]$^+$ 255.1 [**7** +Na]$^+$ 465.2 [**6** + **7**]$^+$	368.3 [**7** + adeH]$^+$	343.1 [**6** + cyto]$^+$ 365.1 [**7** + cyto + Na]$^+$	476.1 [**6** + cyti]$^+$ 498.0 [**7** + cyti + Na]$^+$	538.0 [**7** + guo + Na]$^+$	1620.2 [d(CpGp) + **6**]$^+$ 1642.2 [d(CpGp) + **7** + Na]$^+$ 1874.1 [d(CpGp) + **7** + **7** + Na]$^+$
8	487.0 [**7** + **7** + Na]$^+$ 204.2 [**8**]$^+$ 226.1 [**9** + Na]$^+$ 407.1 [**8** + **9**]$^+$ 429.0 [**9** + **9** + Na]$^+$	N.d.^{a}	314.9 [**8** + cyto]$^+$ 336.2 [**9** + cyto+Na]$^+$	447.0 [**8** + cyti]$^+$ 469.1 [**9** + cyti+Na]$^+$	510.0 [**8** + guo]$^+$	1642.2 [d(CpGp) + **7** + Na]$^+$ 1611.9 [d(CpGp) + **9** + Na]$^+$
10	275.1 [**10**]$^+$ 297.9 [**11** + Na]$^+$ 549.3 [**10** + **11**]$^+$ 571.3 [**11** + **11** + Na]$^+$	N.d.^{a}	407.1 [**10** + cyto + Na]$^+$	540.3 [**10** + cyti + Na]$^+$	581.2 [**10** + guo + Na]$^+$	1684.3 [d(CpGp) + **10** + Na]$^+$

^{a} Not detected.

the uracil derivatives—and one small peak at m/z 368.3 which corresponds to a 1 : 1 adduct of **6** and adenine, or **7** and adeninium. Results are summarized in Table 4.

Complexes between **7** and adenine are not able to reach a point of minimal energy in *ab initio* calculations, even if smaller basis sets (3–21G**, STO-3G**) were applied. The calculations, however, indicated stable hydrogen bonded associates between adeninium adeH[+] and the salt **6**. We took Hoogsteen base pairings **6** = adeH[+] into consideration and calculated the plausible geometries *A*, *B*, and *C* (Scheme 7, Table 5), as the Watson–Crick binding site of adenine is protonated. The Hoogsteen pair **A** is, by 1.405 kcal mol^{−1}, more stable than **B**, whereas **C** was not able to reach a stable minimum. In agreement with these observations, ^{1}H NMR spectra of 1 : 1 solutions of adeninium adeH[+] and **6** in DMSO-d_6 displayed considerable downfield shifts of the N(10)–H ($\Delta\delta$ = 0.37 ppm) and 12-H ($\Delta\delta$ = 0.10 ppm) of the uracil, thus proving horizontal interactions in comparison the ^{1}H NMR spectra of
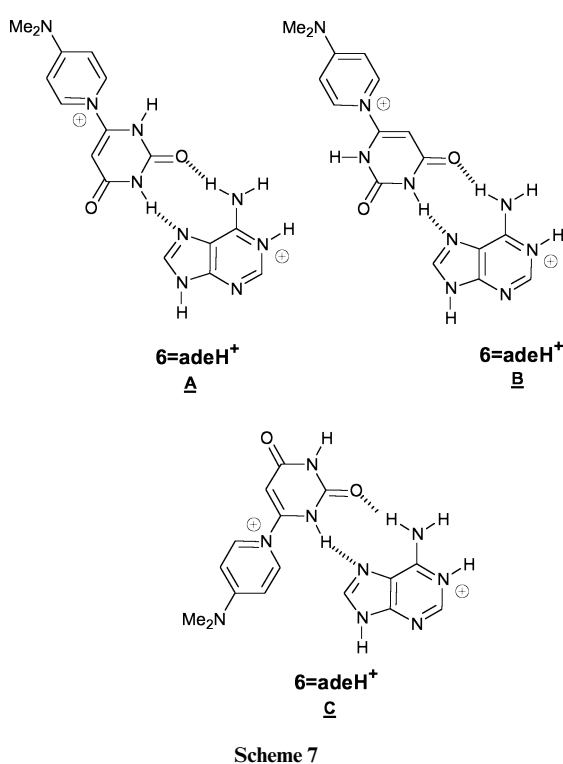
adeH[+] and **6** alone. As expected, acid–base equilibria were found on studying the cation **6** in mixtures with adenine in DMSO-d_6.

In calculations (HF/6-31G**), the geometry *A* of hydrogen bonded dimers between the betaine **7** and cytosine cyto=**7** (−1183.721887 Hartrees) proved to be 0.91535 kcal mol^{−1} more stable than *B* (−1183.7204260 Hartrees) (Scheme 8). Results of our calculations are presented in Table 5. The mass spectra derived from electrospray ionization of anhydrous acetonitrile solutions of 1 : 1 mixtures of the uracilylbetaine **7** with cytosine unambiguously showed base-mispairs which were detectable at m/z 366.1. Likewise, spraying samples of cation **7** under analogous measurement conditions gave prominent peaks at m/z 344.1 which can be attributed to 1 : 1 adducts between **7** and cytosine. In addition, **7**, **7**=**7**, and cyto≡cyto (m/z 223.1) were detected. Analogous results were obtained spraying solutions of betaine **7** and cation **6** with cytidine, the N1-position of which is blocked by the ribose moiety. Prominent peaks of 1 : 1 adducts were detected (Table 4).

6=adeH[+]
A

6=adeH[+]
B

6=adeH[+]
C

Scheme 7

cyto=7
**A**

cyto=7
B

Scheme 8
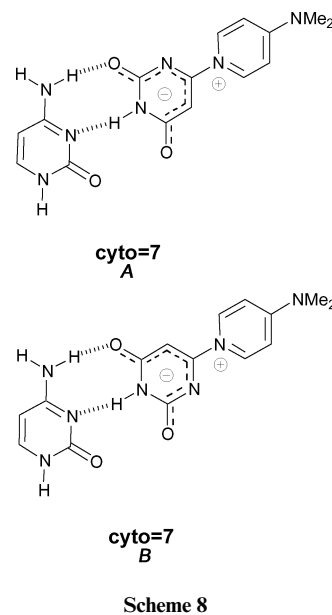
Next, we turned our attention to base-pairings with guanosine, which we used in ESI mass measurements instead of guanine, as it is more soluble in water–acetonitrile solutions. Again, we found peaks of 1 : 1 adducts at m/z 538.0, although in low intensity.

Table 5 Calculated torsion angles and hydrogen bonding parameters of adeH[+]=**6**, cyto=**7** and gua=**7**

	Torsion angle between molecules (deg)	N–H…O parameters	N–H…N parameters
6=adeH[+] (*A*)	0.5	284 pm 166.5°	286 pm 178.3°
6=adeH[+] (*B*)	2.6	279 pm 166.2°	287 pm 179.0°
cyto=**7** (*A*)	24.0	275 pm, 178.7°	306 pm 169.6°
cyto=**7** (*B*)	19.6	277 pm, 178.5°	308 pm 168.2°
gua=**7** (*A*)	1.6	Central: 332 pm, 169.9° Lateral: 275 pm, 178.7°	—
gua=**7** (*B*)	1.5	Central: 300 pm, 173.2° Lateral: 270 pm, 169.3°	—

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Calculations on base-pairings of **7** and guanine show that complex **A** of gua=**7** is more stable than complex **B** ($\Delta = 1.40694$ kcal mol^{-1}) (Scheme 9).


gua=7
A

gua=7
B
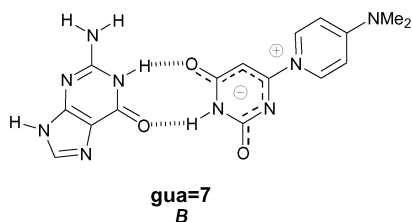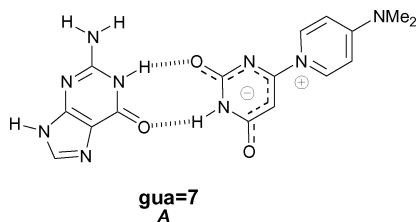
Scheme 9

These findings encouraged us to examine d(CpGp) as a base partner. Indeed, spraying a 1 : 1 solution of the betaine and d(CpGp) in aqueous acetonitrile from aqueous acetonitrile gave a mass spectrum with the peaks of monomeric d(CpGp) as sodium adduct, of d(CpGp) associated with the betaine **7** (m/z 1620.2), the corresponding sodium adduct (m/z 1642.2) and the associate of two betaine molecules and d(CpGp) as sodium adduct at m/z 1874.1. Proposed structures, based on the aforementioned results and calculations, are shown in Scheme 10. Due to the similar energies of the two cyto=**7** complexes presented in Scheme 8, the alternative geometry must also be taken in consideration. The salts **8** and **10**, and the betaines **9** and **11** were examined analogously and showed a very similar behaviour toward nucleobases. All mass spectrometric results are summarized in Table 4.
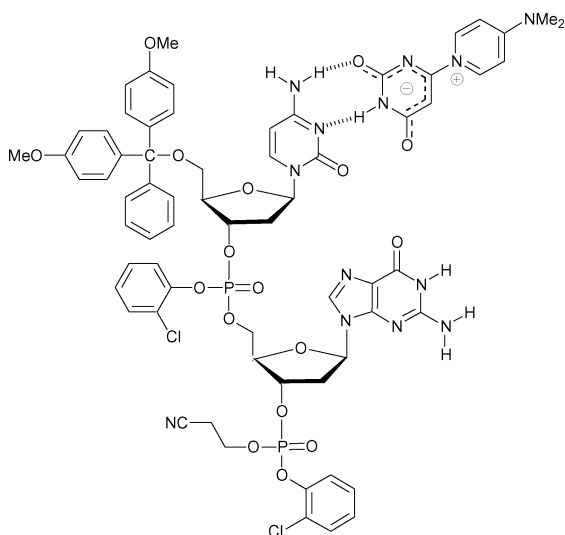
In summary, we present modified uracils which belong to the class of cross-conjugated heterocyclic mesomeric betaines (CCMB). These betaines possess interesting π-stacking and base-pairing properties in comparison with the non-modified nucleobase uracil.
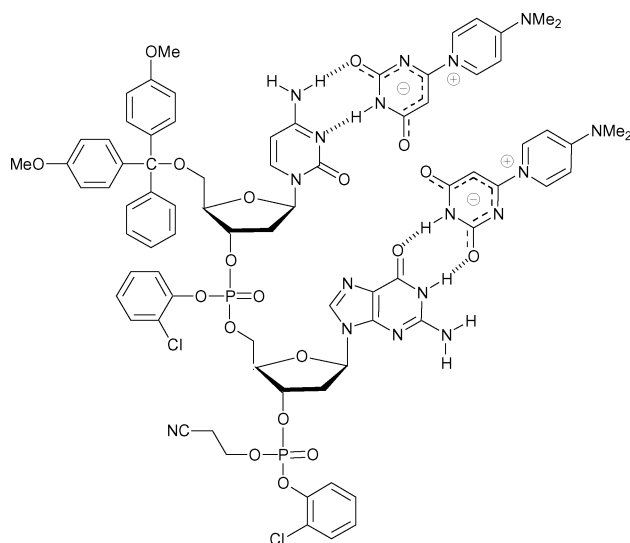
Scheme 10

Experimental

General remarks

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Digital FT-NMR Avance 400 and Avance DPX 200 spectrometers. Multiplicities are described by using the following abbreviations: s = singlet, d = doublet, m = multiplet. The numbering is defined in Scheme 2. FT-IR spectra were obtained on a Bruker Vektor 22 in the range of 400 to 4000 cm^{-1} (2.5% pellets in KBr). The electrospray ionisation mass spectra (ESIMS) were measured with an Agilent LCMSD Series HP1100 with APIES. Measurement conditions are given in Table 4. Melting points are uncorrected. The compounds **6** and **8** were prepared as previously described.[26] Quantum chemical calculations were performed using the GAUSSIAN-03 package of programs.[50] We always used the split-valence 6-31G** basis set,[51,52] which includes six s-type and three p-type polarization functions on all the atoms. Electron correlation energy was introduced using the hybrid functional B3PW91, within the density functional theory (DFT).[53,54] The minimal energy geometry, the topology of the frontier orbitals, the natural bond orders (NBO) and the electrostatic surface potential of the 4-(dimethylamino)pyridine substituted uracils **6** and **7** were computed by simulating a polar environment by means of a polarizable continuum model (PCM).[55,56] In this model the solute molecule is placed into a size-adapted cavity formed from overlapping atom-centred van der Waals spheres, while the solvent is assimilated to a continuum characterized by its dielectric constant (78.4 for water). Electronic excitation energies were obtained for the optimized structures of **6** and **7** by using the time-dependent (TD) formalism,[57,58] for which the fifteen states of lower energy were considered. The theoretical studies of hydrogen bonding were performed on gas phase systems. We used the 6-31G** basis set in combination with both DFT (B3PW91) and HF calculations, to compare results. The following general procedure was used:

(i) Optimization of the isolated molecules at the same level of calculation used for the hydrogen bonding complexes.

(ii) Building of the complexes by hydrogen bonding docking, using standard parameters.

(iii) Optimization of the complexes as a whole, thus allowing all the geometrical parameters to vary independently.

Crystal structure determination of 10 and (ade(H⁺)₂ 2 Cl⁻)‡

10. Crystal data. $C_{13}H_{17}ClN_4O_4$, [$(C_{13}H_{15}N_4O_3)^+$ Cl^-·H₂O], $M = 328.76$, monoclinic, $a = 6.9530(1)$ Å, $b = 7.9824(2)$ Å, $c = 26.3131(6)$ Å, $\beta = 92.122(1)°$, $U = 1458.86(5)$ Å³, $T = 123(2)$ K, space group $P2_1/n$ (no. 14), $Z = 4$, μ(Mo Kα) = 0.287 mm⁻¹, 7161 reflections measured, 3239 unique ($R_{int} = 0.0229$) which were used in all calculations. The final $wR(F2)$ was 0.0820 (all data) with $R1 = 0.0296$ for $I > 3\sigma(I)$.

(ade(H⁺)₂ 2 Cl⁻)‡. Crystal data. $C_5H_7Cl_2N_5$, [$(C_5H_7N_5)_2^+$ 2Cl⁻], $M = 208.06$, orthorhombic, $a = 13.4485(12)$ Å, $b = 6.4673(6)$ Å, $c = 9.3711(6)$ Å, $U = 815.06(12)$ Å³, $T = 123(2)$ K, space group $Pnma$ (no. 62), $Z = 4$, μ(Mo Kα) = 0.744 mm⁻¹, 3163 reflections measured, 997 unique ($R_{int} = 0.0356$) which were used in all calculations. The final $wR(F2)$ was 0.0717 (all data) with $R1 = 0.0265$ for $I > 3\sigma(I)$.

General procedure for the synthesis of the uracilium salts 8 and 10

A suspension of 6-chlorouracil (0.73 g, 5.0 mmol) and 5.0 mmol of the heteroaromatic [4-methylpyridine (0.47 g), 4-morpholino-pyridine (0.82 g)] in 150 mL of chlorobenzene was heated at reflux temperature over a period of 3 h. After cooling, the precipitate was filtered off, washed with 30 mL of dichloromethane, and recrystallized from ethanol, water and hydrochloric acid (10 : 5 : 1) in the presence of charcoal.

4-Methyl-1-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-6-yl)pyridinium chloride (8)

Slightly brownish solid, yield 0.65 g (55%), mp 250 °C (dec.) (found: C, 47.39; H, 3.79; N, 16.86. $C_{12}H_{14}N_3O_2$·0.75H₂O requires C, 47.44; H, 4.57; N, 16.59); δ_H 2.73 (s, 3H; Me), 6.25 (s, 1H; 12-H), 8.21 (d, $J = 6.6$ Hz, 2H; H$_{ar}$), 9.18 (d, $J = 6.6$ Hz, 2H, Har), 11.71 (s, broad, 1H; NH); δ_C 22.1, 98.2, 127.9, 143.1, 149.7, 150.1, 163.1, 164.0; ν_{max} (KBr)/cm⁻¹: 3423, 3164, 3049, 2826, 1686, 1639, 1501, 1472, 1414, 1350, 1282, 1212, 1093, 1024, 985, 860.

4-(4-Morpholino)-1-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-6-yl)pyridinium chloride (10)

Colorless solid, yield 0.25 g (16%), mp 290 (dec.) (found: C, 47.34; H, 4.89; N, 17.32. $C_{13}H_{15}N_4O_3Cl$·H₂O requires C, 47.49; 5.21; N, 17.04); δ_H 3.77 (m, 4H), 3.83 (m, 4H), 6.02 (s, 1H; 12-H), 7.39 (d, $J = 7.7$ Hz, 2H; H$_{ar}$), 8.46 (d, $J = 7.7$ Hz, 2H; H$_{ar}$), 11.53 (s, 1H; NH). δ_C 46.9, 65.5, 95.4, 107.4, 140.4, 149.4, 150.6, 159.1, 163.5 ppm; ν_{max} (KBr)/cm⁻¹: 3491, 3442, 3062, 2693, 1717, 1673, 1649, 1549, 1524, 1408, 1275, 1107, 1028, 932.

General procedure for the synthesis of the mesomeric betaines 9 and 11

30 g of the anion exchange resin Amberlite IRA-400 was filled into a column and treated with 8% NaOH over a period of 30 min. Then, the resin was washed with water until pH 7 of the elute was

measured. The salts **8** (0.2 g; 0.84 mmol) and **10** (0.1 g, 0.32 mmol) were dissolved in water, poured onto the resin, and eluted with water, respectively. The elutes were collected and evaporated to dryness, whereupon white solids were obtained.

4-Methylpyridiniopyrimidine-(1H,3H)-2,4-dionate (9)

Yellow solid, yield: 0.086 g (98%), mp 290 °C (dec.) (found: C, 31.74; H, 3.97; N, 11.59; $C_{13}H_{14}N_4O_3$·11H₂O requires 31.84; H, 7.81; N, 11.42); δ_H 3.74 (m, 4H), 3.75 (m, 4H), 5.93 (s, 1H; 12-H), 7.09 (d, $J = 8.12$ Hz, 2H; Har.), 8.12 (d, $J = 8.12$ Hz, 2H; Har.) ppm; δ_C 46.9, 65.5, 95.4, 107.4, 140.4, 149.4, 150.6, 159.1, 163.5 ppm; ν_{max} (KBr)/cm⁻¹: 3423, 3217, 1625, 1541, 1460, 1414, 1378, 1273, 1205, 1107, 1054, 1016, 928, 813.

4-(Morpholin-4-yl)pyridiniopyrimidine-(1H,3H)-2,4-dionate (11)

Brownish solid, yield: 0.044 g (26%) due to partial decomposition on the column, mp. 265 °C (dec.) (found: C, 29.49; H, 3.49; N, 10.08. $C_{10}H_9N_3O_2$·11H₂O requires C, 29.92; H, 7.78; N, 10.47). δ_H 2.68 (s, 3H; Me), 5.71 (s, 1H; 12-H), 8.02 (d, $J = 6.72$ Hz, 2H; Har.), 9.33 (d, $J = 6.72$ Hz, 2H; Har.), 10.06 (s, 1H; 10-H) ppm; δ_C 21.6, 86.3, 127.8, 140.1, 161.9, 166.7 ppm; ν_{max} (KBr)/cm⁻¹: 3406, 1629, 1484, 1406, 1363, 1285, 1240, 1195, 1163, 986, 809.

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